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# THE EPIDEMIOLOGY OF HEMOLYTIC STREPTOCOCUS CARRIERS, BASED ON A STUDY OF SUBGROUPS \*

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The hemolytic streptococci came into great prominence during the World War, as they were one of the most common causes of death in the respiratory diseases. In the investigation of the possible modes of spread of the streptococcus as complicating these infections numerous studies have been made on the incidence of carriers. However, most of the carriers thus diagnosed have been identified as harboring organisms belonging to the hemolytic streptococcus group as a whole rather than to any of the subgroups. Advance in bacteriology and epidemiology rests on the subdivision of a group of organisms, as in the case of the pneumococci. In this paper an attempt is made to approach the problem of epidemiology through such a study of subgroups. Subdivisions of hemolytic streptococci based on their fermentation reactions, is possible and is of value, since striking and suggestive results were obtained by grouping carriers and by similarly grouping organisms obtained from the air of hospital wards—in other words, from the environment.

The serologic subgrouping of streptococci has given suggestive results in the hands of Havens,<sup>1</sup> and of Dochez and his co-workers,<sup>2</sup> but on account of technical considerations it was not felt advisable to attempt this method of classification for the purposes of this paper.

All the strains studied in this paper were isolated at Columbus Barracks, Ohio, during February and March, 1920. The fermentation reactions were done at the Army Medical School. Columbus Barracks was selected as the place of making the survey, since it is a recruit depot and afforded opportunity for the comparison of the incidence of carriers among men arriving from civil life with that prevailing among the permanent personnel at the post. It was also possible to reculture recruits after a brief period in the service in

<sup>1</sup> Jour. Infect. Dis., 1919, 25, p. 315.

<sup>2</sup> Dochez, Avery and Lancefield, Jour. Exper. Med., 1919, 30, p. 179.

order to ascertain whether conditions prevailing at an army post during peace time caused any increase in the carrier incidence. The general data thus obtained was felt to be of enough interest to warrant its inclusion in the paper. The Barracks had at the time of this work a soldier population of approximately 1,300. An average of 40 to 50 recruits were being enlisted daily.

#### TECHNIC

The technic<sup>3</sup> made official for army use during the war was followed. Sterile swabs were rubbed with moderate pressure over the tonsils or fossae. These were spread over the surface of blood agar plates within 20 minutes and incubated. Beef infusion agar was used,  $P_H$  7.6, to which 10% of its volume of defibrinated human blood was added. Twelve to fifteen cc of medium were used per plate. On the following morning hemolytic colonies were transferred to beef infusion broth, which after 24 hours' growth were tested for hemolytic activity by mixing 0.5 cc of the broth culture with an equal volume of a 5% suspension of washed blood cells and placing in water bath at 37 C. for 2 hours.

The hemolytic character of the organisms was later confirmed, using pour plates made of rabbit blood. With the exception of a few strains which were lost or not saved for further study, all the strains considered in this work were shown to produce the beta type of hemolysis, as described by Brown.<sup>4</sup>

The question arises as to the value of positive and negative results as detected by the technic described. Davis<sup>5</sup> believes that practically everybody harbors the hemolytic streptococcus. If this is correct, a negative result would have much less significance than otherwise. However, Nichols and Bryan,<sup>6</sup> using the same method as Davis, that is, the culturing of tonsils that had been removed, found hemolytic streptococci in only 75% of cases. Ruediger,<sup>7</sup> in 1906, stated that while the hemolytic streptococcus was frequently found in the throat, it could not be regarded as a normal inhabitant. This statement, I believe,

<sup>3</sup> Methods for the Isolation and Identification of *Streptococcus Hemolyticus*, U. S. Army, 1918.

<sup>4</sup> Monographs of the Rockefeller Institute for Medical Research, 1919, No. 9.

<sup>5</sup> Jour. Am. Med. Assoc., 1920, 74, p. 317.

<sup>6</sup> Ibid., 1918, 71, p. 1813.

<sup>7</sup> Jour. Infect. Dis. 1906, 3 p 755.

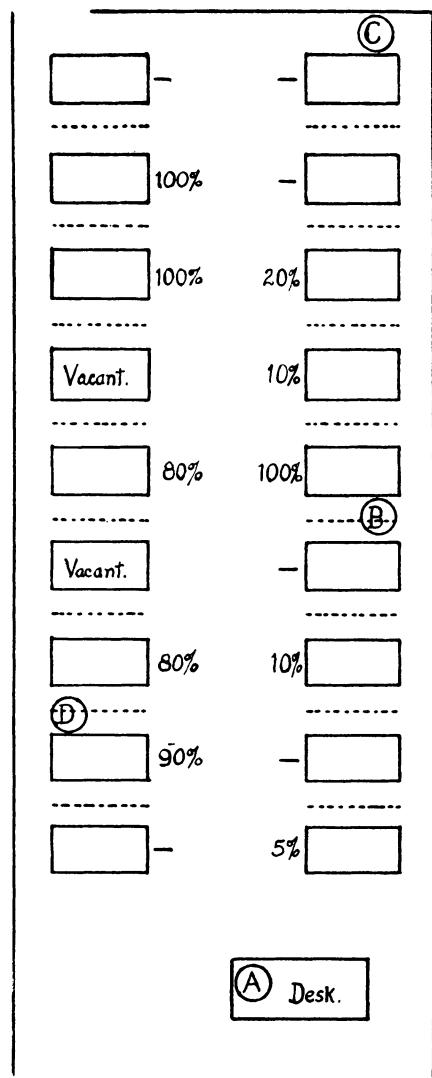


Diagram of ward 1, showing the relationship of positions in which blood agar plates were exposed to patients. The dotted lines represent screens, which were used to cubicle the ward. Approximate Scale: 1 inch = 12 feet.

still represents our present knowledge of the subject. Although it is true that some lightly infected cases may fail to be detected, this fact does not invalidate the method as a whole.

A word of caution should be said in regard to a possible error if one attempts to identify the hemolytic streptococcus simply from the appearance of a throat culture on a blood-agar plate. We have frequently encountered a hemolytic bacillus, the colonies of which on rabbit blood closely resemble those of typical hemolytic streptococci. This organism seems to be strictly hemoglobinophilic, and possibly belongs to the *B. influenzae* group, several hemolytic strains of which have been described by Rivers.<sup>8</sup> The organism is considerably larger, however, than the *B. influenzae* usually encountered.

The classification of Holman<sup>9</sup> was followed in the fermentation reactions. He classified the strains encountered by us thus:

	Lactose	Mannit	Salicin
St. infrequens .....	+	+	+
St. pyogenes .....	+	-	+
St. anginosus .....	+	-	-

All strains were also tested against inulin. Only one inulin fermenter was encountered in the total of 203 strains tested. This organism grew in long chains, was bile insoluble, and produced no capsules. Otherwise it fermented as *St. pyogenes*.

The medium used in the fermentation tests was horse serum 1 part, distilled water 4 parts, to which was added 1% of Andrade indicator and 1% of the test sugar. After the sugar was dissolved, the medium was tubed and autoclaved for 10 minutes at 10 pounds' pressure. Streptococci grew well in this medium, even before the addition of the test substances.

Before being tested, all strains were plated and single colonies transferred to broth. The fermentation tubes were inoculated with 0.1 c.c. of the broth cultures and incubated 24 hours. A preliminary reading was then made, and smears made from each tube on blood-agar plates. Tubes and plates were then returned to the incubator. No results were considered unless the blood-agar plate showed the profuse growth of a streptococcus in pure culture. In case of lack of growth or of contamination the test was repeated. Final readings were made on the seventh day.

<sup>8</sup> Bull. Johns Hopkins Hosp., 1920, 31, p. 50.

<sup>9</sup> Jour. Med. Res., 1916, 34, p. 377.

The organisms from the air were isolated by the exposure of blood-agar plates, for known periods of time. These plates were placed on desks or bedside tables. More improved methods of air examination have, of course, been described, but the results furnished by this method are believed to be valuable, besides having the advantage of great simplicity.

#### RESULTS OF CULTURES

Throat cultures were made on recruits as they arrived at the barracks and as they left. The length of stay at the barracks varied from 5 to 13 days. Cultures were similarly made from the throats of a group of soldiers from the permanent personnel of the post. The latter group all had had one or more years' service. The results are shown in table 1.

TABLE 1  
INCIDENCE OF STREPTOCOCCUS CARRIERS AMONG RECRUITS AND AMONG PERMANENT PERSONNEL

	Number Cultured	Number Positive	Proportion, Percentage
Recruits arriving.....	470	79	17
Recruits leaving.....	175	28	16
Permanent personnel.....	99	20	20

It is seen from this table that the incidence of carriers among recruits having spent a short period at the post shows no increase over the rate prevailing among them on arrival. These rates are slightly less than that among the permanent personnel of the post, but the difference is not believed to be of significance. The incidence of 17% shown by the recruits arriving for enlistment can perhaps be taken as an index of the civilian rate. On this basis it is seen that the carrier ratio at the post was approximately the same as that among civilians.

It is interesting to compare these figures with the work of Levy and Alexander,<sup>10</sup> who found that recruits arriving at Camp Zachary Taylor (near Louisville, Ky.), in 1918, showed a carrier incidence of only 14.8%, while a group of men who had been at the camp 6 months or more showed an incidence of 83.2%. The figures obtained at Columbus show, however, that such an increase is not an invariable rule. The greater proportion of the recruits examined at Columbus (79%) were from urban communities.

<sup>10</sup> Jour. Am. Med. Assoc., 1918, 70, p. 1827.

The incidence of carriers among those with abnormal throats was higher than among the rest of the group; 34% of those showing hypertrophied tonsils or injection of the pharynx were positive. The influence of pathologic conditions of the throat were especially marked in relation to the persistence of positive cultures. Of the 175 men cultured twice, 163 were considered as having normal throats. Of these seven, or 4%, were positive on both occasions. The remaining 12 were noted as having hypertrophied tonsils or injection, or both. Five of them, or 38%, were positive on the 2 cultures. Ten cases had had previous tonsillectomies, all of whom were negative for hemolytic streptococci.

TABLE 2  
CLASSIFICATION OF THE HEMOLYTIC STREPTOCOCCI ACCORDING TO THEIR FERMENTATION REACTIONS

Source	Number of Strains	Pyogenes		Infrequens		Anginosus	
		Num-ber	Proportion, Percentage	Num-ber	Proportion, Percentage	Num-ber	Proportion, Percentage
Recruits arriving.....	65	53	82	10	15	2	3
Recruits leaving.....	27	23	85	4	15	0	
Permanent personnel.....	20	13	65	1	5	6	30
Hospital detachment.....	7	6	86	0	0	1	14
Measles.....	14	6	43	8	57	0	
Other patients.....	32	30	94	2	6	0	
Air.....	34	30	88	4	12	0	
Total.....	199	161	80	29	15	9	5

The rest of the organisms isolated from throats were obtained from a survey of 37 men on duty at the hospital, of whom 9 (24%) were positive, and from 81 patients in the hospital, of whom 48% were positive. Among the latter the highest incidence was in 17 cases of measles, of whom 14, or 82%, were positive, and 3 cases of scarlet fever, all of whom were positive. The lowest percentage of carriers was in a ward of 18 A. E. F. patients with facial wounds. These showed an incidence of 11%.

The classification of these organisms and those obtained from the air is shown in table 2.

Compared with the recruits, the permanent personnel and the hospital detachment show a high percentage of anginosus strains. Thirty per cent of the carriers among the permanent personnel were infected with anginosus and none of the recruits leaving had this strain. This

makes it probable that any new carriers produced among the recruits during their stay at the Barracks were infected from their companions rather than from the men permanently on duty at the post.

The anginosus subgroup is also conspicuous by not occurring among the strains isolated from patients. At Columbus, at least, it could be practically disregarded as a cause of disease.

The high percentage of St. infrequens occurring in measles patients is striking. It is possible that this resulted simply from new patients with measles becoming infected with the strains of infrequens present in the ward, rather than to special predilection of these organisms for patients with measles.

The infrequens strains from two other patients (table 2) came, one from a case of tonsillitis and the other from bronchopneumonia following measles. Since the remaining strains from patients were all pyogenes, it is not necessary to give in detail the conditions in which they occurred.

The following instances are cited since they tend to show that an organism causing an infection or a carrier state in an individual remains true to type: As mentioned, 12 recruits were positive on both the occasions that they were cultured. The subgroups of the strains isolated from each coincided exactly on the two cultures, 10 of them being pyogenes and 2 infrequens.

Two of the measles cases, which were complicated with otitis media, furnished information of the same nature. The strains from the throat and from the ears were in both instances infrequens. Another patient in the surgical ward had an infected appendectomy wound, from which St. pyogenes was isolated, as well as from his throat.

The question of the persistence of a subgroup of the organism in an individual is of great importance in relation to the epidemiology of streptococcus infections and has a direct bearing on all efforts to trace the source of an infection. From the instance cited, for example, we are justified in concluding that the patients in the measles wards infected with St. pyogenes had no relation to the measles patients who developed otitis media, since the latter were caused by St. infrequens. Also, as far as the evidence goes, it is possible that the infection of the surgical wound was caused by the strain in the patient's own throat. This possibility of course could not be considered had the strains from the throat and from the wound belonged to different subgroups.

## ORGANISMS RECOVERED FROM THE AIR

The presence of a large number of patients in the hospital with positive throat cultures offered opportunity to investigate the occurrence of hemolytic streptococci in the air. A cultural survey was made on the patients at the same time the plates were exposed. Other colonies developing at 37 C. in 24 hours were counted, as well as the hemolytic streptococci.

Table 3 shows the places in which plates were exposed and the number of colonies developing. As a matter of interest, the calculation for the number of hemolytic streptococci settling per hour per square meter is included in the table.

TABLE 3  
STREPTOCOCCI ON BLOOD-AGAR PLATES EXPOSED IN VARIOUS PLACES

Place	Number of Patients	Number of Carriers	Number of Plates Exposed	Time Each Plate Was Exposed, Hours	Total Number Colonies	Total Number of Colonies of Hemolytic St.	Hemolytic St. per Sq. Meter per Hour
Ward 1, medical.....	16	10	16	4	5,735	59	144
Ward 11, venereal.....	6	1	2	2	550	6	236
Ward 2, surgical.....	18	5	3	2	420	5	131
Ward 6a, surgical.....	4	1	2	2	160	2	79
Ward 7, surgical.....	20	1	2	2	375	1	39
Ward 7, surgical*.....	19	0	8	4	2,885	0	

\* Exposure 3 days after carrier at first present in ward had been discharged.

In an experiment by O. Teague, similar to the above, reported by Nichols,<sup>11</sup> the rate of settling out in a ward containing carriers was 8 hemolytic streptococci per square meter per hour. However, his plates were exposed at night after all the patients had retired. The plates mentioned in table 3 were exposed in the afternoon between 1 and 5 o'clock, during which time there was considerable moving about due to the ward routine, and especially to the serving of dinner and supper. This may account in part for the much higher figures obtained at Columbus.

The organisms isolated had the same cultural and morphologic characteristics as organisms isolated from carriers or patients. They belonged to the same subgroups as the organisms from patients. Furthermore, they occurred in greatest number in wards in which there were carriers, and were absent or relatively few in number in

<sup>11</sup> Jour. Lab. & Clin. Med., 1920, 5, p. 502.

a ward in which there were no carriers. These considerations make it entirely reasonable to assume that the organisms from the air were identical with the organisms occurring in the patients.

From table 2 it will be noted that 4 of the organisms obtained from the air were infrequens. These were all from the same ward (ward 11) and included all the organisms recovered from this ward that were tested against the sugars. Only one carrier was detected in this ward, and his strain was St. pyogenes. Unfortunately, cultures were not made from the throats of the ward personnel. Hence, it is believed that in all probability there was a carrier of St. infrequens among the personnel. However, even in the absence of having discovered the origin of these strains, their presence all in one ward is believed to emphasize the practical importance, from the standpoint of epidemiology, of grouping streptococci according to their fermentation reactions.

TABLE 4  
HEMOLYTIC STREPTOCOCCI FROM THE AIR IN WARD 1

Position	March 20		March 21		March 22		March 23		Total Hemo-lytic St.	Total Other Col- nies
	Hemo- lytic St.	Other Col- nies								
A	4	210	5	400	5	550	2	430	16	1,590
B	4	220	2	450	4	450	0	250	10	130
C	7	200	4	550	0	325	2	250	13	134
D	5	250	8	500	2	250	5	550	20	1,550

All the other organisms isolated from the air were pyogenes.

It is advisable to consider ward 1 in some detail. The illustration contains a diagram of the ward. Each bed was cubiced by means of a sheet on a movable frame, which extended about 3 feet above the level of the bed. A minus sign next to a bed means that the patient occupying it had a negative throat culture. The figures in percentage next to the other beds, except those unoccupied, indicate the proportion of the total number of colonies on the throat cultures that were hemolytic streptococci. The positions in which blood-agar plates were exposed are indicated by capital letters enclosed in circles.

Plates were exposed in each position daily for 4 hours over a period of 4 days. A case of scarlet fever had developed in this ward a few days previously, hence the ward was under quarantine and no changes occurred in the patients. The results are shown in table 4.

An analysis of this table shows that hemolytic streptococci were generally distributed throughout the air of the wards, and that cubicles did not prevent their free circulation. Position "C" is the farthest removed from any of the carriers, yet the number of colonies of hemolytic streptococci recovered here is practically the same as in the other positions.

The presence of the organisms in the air may have been due to bacteria or droplets containing bacteria emitted by the patients, or these droplets—saliva—and mucus may have settled to the floor, dried, and later passed into the atmosphere as dust. Possibly the latter factor played the major part, since this would perhaps more satisfactorily explain the general distribution. The presence of hemolytic streptococci in the dust of floors has been noted by several observers. Alexander<sup>12</sup> found them in sweepings from the floors of barracks or hospital wards, as did also Cummings and Spruit.<sup>13</sup> The latter observers, however, were unable to demonstrate them at a level more than 2 inches above the level of the floor. It is perhaps reasonable to assume, if hemolytic streptococci exist on the floor, or especially 2 inches from the floor, that they will, under certain conditions, be carried into the general atmosphere as dust.

The ease with which streptococci spread from the throat of one patient to others has been noted by several observers, especially Cole and MacCallum<sup>14</sup> and Levy and Alexander.<sup>15</sup> The latter studied the problem carefully and found that, in order to keep the throats of measles patients who entered the hospital with negative cultures from becoming positive, it was necessary to put them in separate wards. When patients with negative cultures were put in wards with patients having positive throat cultures many of them became infected, despite the most rigid precautions, including cubicing, masking when not in cubicle, careful watching, discipline, etc. It is possible that the infection of these patients occurred by means of the air at long range, as distinguished from droplet infection at short distance. In my opinion the ease with which streptococcus infection occurs cannot be explained only on the basis of direct contact of individuals, or indirectly by drinking and eating utensils, or by droplet infection at short range, however important these factors may be.

<sup>12</sup> Jour. Am. Med. Assoc., 1918, 70, p. 775.

<sup>13</sup> Mil. Surg., 1920, 46, p. 391.

<sup>14</sup> Jour. Am. Med. Assoc., 1918, 70, p. 1146.

**SUMMARY AND CONCLUSIONS**

The incidence of carriers at an army post during peace times was not greater than that in civil life. New recruits spending a short period of time at this army post did not show an increase in the carrier ratio.

Pathologic conditions of the pharynx, such as hypertrophied tonsils, tend to prolong the state of a hemolytic streptococcus carrier.

The classification of streptococci, according to their reactions on the sugars, is believed to be of great importance from the standpoint of epidemiology, and should be done in every case in which a more thorough knowledge of the streptococci isolated from patients, carriers or the environment is desired.

It is believed that streptococcus infections cannot be explained on the basis of contact alone. Streptococci were found to be distributed generally in the air of a ward containing patients with positive throat cultures. The presence of these organisms in the air probably explains the ease with which streptococcus infections take place. Their number is also probably influenced by the amount of dust in the room. This reemphasizes the necessity of measures to allay dust and of keeping patients with negative throat cultures in separate wards.